Journal of the Washington Academy of Sciences.
Volume 72, Number 1, Pages 1-11, March 1982

The Ionic Basis of Fertilization

David Nishioka

Department of Biology, Georgetown University,
Washington, D.C. 20057 USA

ABSTRACT

Fertilization is considered in terms of two cellular activations. Experiments with sea urchins gametes in which the ionic composition of the external medium is controlled, show that extracellular sodium ions (Na^+) are required for the activations of both the sperm and the egg. These requirements are for the release of intracellular protons (H^+) and result in increased intracellular pH (pH_i). Evidence is presented showing that these changes occur spontaneously upon suspension of sperm in sea water and result in the initiation of sperm motility. In the egg, these changes occur after fusion with sperm and result in the initiation of embryonic development.

Introduction

How cells go from the metabolically repressed to the metabolically active states and how they go from the nondividing to the dividing states have long been recognized as central questions in our understanding of cells. They are crucial to our understanding of multicellular organisms as well. The immune response, wound healing, carcinogenesis, all involve these cellular transformations. In fact, as this review and the three which follow point out, the multicellular state in many organisms is itself predicated on these types of cellular transformations. The questions are clear in the study of fertilization. How does the differentiated sperm cell which is stored in a metabolically repressed state undergo activation to an actively respiring, fully motile state prior to fertilization; and how does the egg, upon fusion with sperm, undergo transformation from the nondividing to the dividing states leading to the development of a new individual?

An implicit concept used in answering these questions is that these cells are closely linked with their environments in predictable and specific ways. It would be difficult to explain the activation of sperm in any other terms. The sperm cell has a limited amount of energy. If it does not fuse with an egg in a limited amount of time, it simply expends this energy and dies. Adult male organisms must provide an environment which preserves this energy until sperm are released. The composition of the external medium then becomes the important limiting factor in whether or not the sperm is activated. The activation of the egg is less direct. An unfertilized egg may remain suppressed for extended periods of time in a medium fully capable of supporting activation. Fusion with sperm is necessary for the activation to proceed, but as for sperm, the composition of the external

medium then becomes the limiting factor. The central questions become more clear. How are these cells linked with their environments to bring about cellular activation?

In this review studies will be presented suggesting that this linkage is provided by specific ions which cross the plasma membranes of these cells. These ionic flows result in changes in the ionic composition of the intracellular environment where they are translated into changes in cell metabolism. For reasons that are obvious, the organism of choice in making these determinations has been the sea urchin. A single female, upon the gentle request of injecting isosmotic KCL into the body cavity, may shed as much as twenty mls of eggs, each ml containing two million eggs. A single male may shed as much as five mls of undiluted semen, each ml containing 10–100 billion sperm. *In vitro* fertilization is accomplished simply by mixing sperm and eggs together in sea water. A most important advantage of this system in determining the involvement of extracellular ions in fertilization is that the ionic composition of the external medium, sea water, is known and may be totally defined. Fertilized eggs will reach first cell division approximately 90 minutes after insemination. The next four to five divisions occur every 60 minutes and remain synchronous within each embryo and within a given culture. Development proceeds synchronously through easily recognizable embryonic stages (blastula, gastrula) to an early larval stage (pluteus) within three days.

Activation of Sperm Metabolism and Motility

Several early studies were directed at the questions of how sea urchin sperm are repressed in undiluted semen and activated upon suspension in sea water. Gray[1] first described the activation as a "dilution effect," explaining the repression in undiluted semen by simple mechanical crowding of the spermatozoa and the activation as a release from this crowding. In support of this idea, he reported that respiration and motility are stimulated when semen is further diluted with seminal fluid (later confirmed by Hayashi).[2] Forcing a reevaluation of this idea, Rothschild[3] reported that when undiluted semen is experimentally oxygenated the spermatozoa become motile, and when it is deoxygenated motility is again inhibited. These results suggest that the lack of oxygen (or CO_2 anoxia) is the repressive factor in undiluted semen and that oxygenation is at least one of the factors involved in the activation of sperm upon suspension in sea water. CO_2 anoxia can be expected if one views undiluted semen as an extremely dense nonvascularized suspension of living cells. The results of Gray and Hayashi can be explained if, during preparation, the seminal fluid became oxygenated, which could occur in the absence of sperm.

There is no doubt that oxygenation is one of the factors required for the activation. Spermatozoa are not anaerobic. However, it cannot be the only factor involved. If this were the case, suspension of undiluted semen in any oxygenated, isosmotic medium should support activation. The results presented in Table 1 indicate that this is not the case. When undiluted semen is suspended in isosmotic solutions of the major salts of sea water, NaCl, KCl, $CaCl_2$, and $MgSO_4$, sperm motility is inhibited in all solutions except NaCl. Additionally, sperm motility is inhibited in sodium-free, choline-substituted sea water (ONa^+-SW) in which all of the major ions of sea water except Na^+ are present in normal amounts. When Na^+ is added back, motility is stimulated. These results suggest that external sodium, the most plentiful cation in sea water, is specifically involved in the activation of sperm metabolism and motility.

It is now known that sperm release acid upon suspension in sea water[4–6] and that this release is dependent upon extracellular

Table 1—Sperm Motility in Isosmotic Salt Solutions[a]

Solution[b]	Motility[c]
ASW	+
NaCl (0.51 M)	+
KCL (0.51 M)	−
$CaCl_2$ (0.34 M)	−
$MgCl_2$ (0.34 M)	−
$MgSO_4$ (0.51 M)	−
ONa^+-SW	−
ONa^+-SW + 0.01 M NaCl	+

[a] 5.0 μl of undiluted semen added to 10.0 ml of solution with constant stirring.

[b] ASW (artificial sea water) is composed of 0.423 M NaCl, 0.009 M KCl, 0.00927 M $CaCl_2$, 0.02294 M $MgCl_2$, 0.0255 M $MgSO_4$, 0.00215 $NaHCO_3$, pH 8.0. Osmolarity = 1.02 OsM.
All salt solutions buffered at pH 8.0 with 0.01 M tris. ONa^+-SW (Sodium-free sea water) is prepared according to the same formula for ASW substituting choline chloride for NaCl and $KHCO_3$ for $NaHCO_3$.

[c] Scored as motile (+) or immotile (−) 30 seconds after suspension.

sodium.[6] As shown in Figure 1a, it can be observed as a drop in pH of the suspension, and is composed of two kinetically different components, one fast which immediately lowers the pH of the suspension from 8.0 to 7.6, and one slow which further lowers the pH to 7.4 over the three minute time period monitored. The respiratory inhibitor cyanide completely inhibits the slow component, while the fast component is unaffected (Figure 1b). These results confirm that the release is composed of two components, i.e., they are not only kinetically separable but also experimentally separable; and they indicate that the slow component represents increased respiration and production of metabolic acid (CO_2). The latter indication is supported by the fact that motility is inhibited in the presence of cyanide. Neither component is observed in ONa^+-SW in which motility is

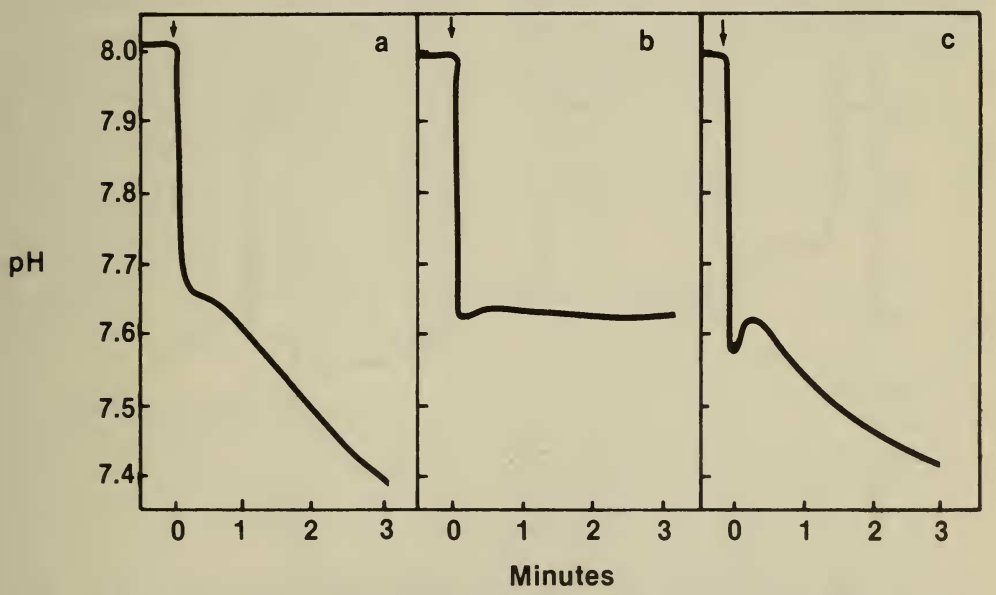


Fig. 1. Acid release from sea urchin sperm. [a] 250 μl undiluted semen added to 2.0 ml ASW at 0 time (arrow). [b] 250 μl undiluted semen added to 2.0 ml ASW containing 10^{-4} M potassium cyanide at 0 time (arrow). [c] 250 μl undiluted semen washed and resuspended in 2.0 ml ONa^+-SW; at 0 time (arrow) NaCl, q.s. 10 mM, was added. The pH of these suspensions (extracellular pH) was measured continuously with a Corning Model 12 expanded scale pH meter equipped with a Fisher Microprobe combination electrode and a Fisher Series 5000 chart recorder.

inhibited and the pH of the suspension remains constant at 8.0. When sodium is added back, the normal two-component release proceeds (Figure 1c), and motility is stimulated.

The picture which has emerged from these facts is that the fast component represents a rapid exchange of extracellular Na^+ into the cell for intracellular protons (H^+) out, and the slow component represents increased metabolism. Viewed in this way, the separability of the two components becomes more important. Since the fast component precedes the slow component, it could control metabolism and motility. This idea finds support in the fact that both components of acid release from sperm suspended in ONa^+-SW are proportional to the amount of Na^+ added back (Figure 2). The results presented in Table 2 confirm that the rate of respiration is also directly proportional. It is as if metabolic activation may be titrated with extracellular Na^+. In the presence of cyanide, Na^+-H^+ exchange occurs but metabolism is inhibited at a later link in a chain of events, in the case of cyanide, at the level of the cytochrome system and electron transport.

Table 2—O_2-Consumption by Sea Urchin Sperm in ONa^+-SW[a]

mM Na^+ added	O_2-consumption nM $O_2/2 \times 10^9$ sperm/min
0.0	1.062
0.5	2.165
1.0	4.330
1.5	25.980
2.5	54.125
5.0	64.950
10.0	73.610

[a]Measured with a Yellow Springs Model 5331 oxygen electrode.

An obvious dilemma that the Na^+-H^+ exchange hypothesis raises is whether the metabolic switch is increased intracellular Na^+ or decreased intracellular H^+. It is now becoming clear that the latter alternative is

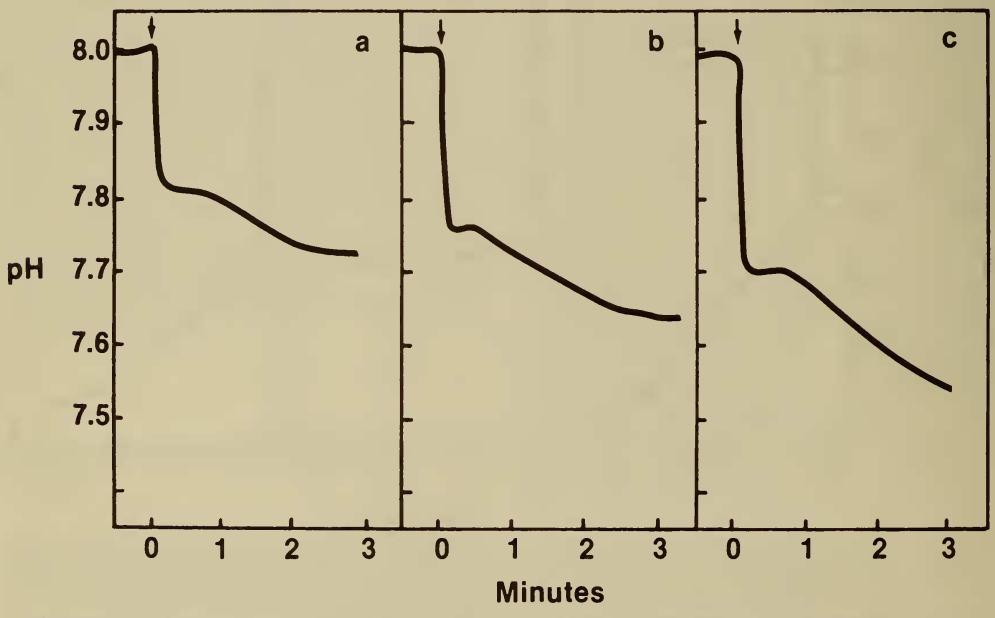
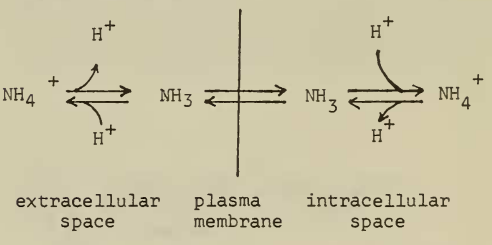


Fig. 2. Acid release from sea urchin sperm. 250 μl undiluted semen was washed and resuspended in 2.0 ml ONa^+-SW. At 0 time (arrows) varying amounts of NaCl, q.s. 0.25 mM [a], 0.5 mM [b], and 1.0 mM [c], were added. Measurements were made as described for Fig. 1.

the important intracellular change. Several methods for measuring intracellular pH (pH_i) in sperm have recently been developed[7-9] and all show increases in pH_i (decreased H^+) which correlate directly with increases in metabolism and motility. These studies agree that upon addition of Na^+ to sperm suspended in ONa^+-SW the pH_i increases 0.4–0.6 pH units, although there is some disagreement about the absolute pH_i values. Lee *et al.* and Lee and Epel (unpublished results) calculate pH_i's of 6.5 in resting sperm and 6.9–7.1 in activated sperm, while Christen *et al.*[9] calculate respective pH_i's of 7.0 and 7.4. Regardless of the absolute values, there is good experimental evidence that the increase stimulates metabolism and motility. For example, Table 3 shows that when the pH of a sperm suspension in ONa^+-SW is raised to pH 9.0 with KOH, sperm motility is stimulated. The OH^- moiety of KOH is the effective variable, since additions of KCl equal to the amount of KOH used to raise the pH have no effect, i.e., the sperm remain immotile. Additionally, K^+ is already present in ONa^+-SW in normal amounts (ca., 0.009 M). When the pH of this same suspension is returned to 8.0 with HCl, motility is again inhibited. In this case the H^+ moiety of HCl is the effective variable, since Cl^- is already present in ONa^+-SW in normal and much larger amounts (ca., 0.496 M) than the amount of HCl used to lower the pH. Thus, sperm motility may be controlled by varying the extracellular pH in ONa^+-SW. These results are interpreted as a bypass of normal Na^+-H^+ exchange and a direct control over pH_i. No Na^+ is present and pH is the only effective variable in the system. This type of control is apparently not possible in normal Na^+-containing sea water in which Na^+-H^+ exchange may occur and sperm motility may be observed throughout the pH range 6.5–9.5. It is interesting to note that the lower limit of this pH range approximates the calculated pH_i's of resting sperm. For the pH_i to be raised to activating levels at this extracellular pH, protons would have to be transported against a concentration gradient.

Another means of bypassing Na^+-H^+ exchange can be achieved by adding NH_4Cl (final concentration, 0.01 M) to sperm suspended in ONa^+-SW. Through a well-studied transmembrane equilibrium, extracellular NH_4^+ raises intracellular pH by equilibrating with NH_3 outside the cell, entering the cell as NH_3, and then re-equilibrating with NH_4^+ inside the cell by taking up intracellular protons.



As shown in Table 3, when NH_4^+ is added to sperm suspended in ONa^+-SW, motility is stimulated. Removal of extracellular NH_4^+ should shift the transmembrane equilibrium in the reverse direction and lower intracellular pH. When NH_4^+-activated sperm are centrifuged and resuspended in ONa^+-SW without NH_4^+ present, motility is again inhibited. The results in Table 4 confirm that the stimulations of sperm motility in ONa^+-SW induced by raising the pH to 9.0 with KOH and by adding 10.0 mM NH_4Cl are coupled with increased respiratory rates.

Table 3—Sperm Motility in ONa^+-SW[a]

Treatment	Motility[c]
Adjusted to pH 9.0 with KOH	+
Readjusted to pH 8.0 with HCl	−
+10.0 mM NH_4Cl	+
$-NH_4Cl$[b]	−

[a] 5.0 μl indiluted semen added to 10.0 ml ONa^+-SW with constant stirring.

[b] Motile sperm in ONa^+-SW + 10.0 mM NH_4Cl centrifuged and resuspended in ONa^+-SW-NH_4Cl.

[c] Scored as motile (+) or immotile (−) 30 seconds after suspension.

Table 4—O_2-Consumption by Sea Urchin Sperm[a]

Medium	O_2-consumption (nanomoles $O_2/2 \times 10^9$ sperm/min)
ASW	88.33
ONa^+-SW	1.06
ONa^+-SW + 10.0 mM NaCl	73.61
ONa^+-SW, pH 9.0	56.29
ONa^+-SW + 10.0 mM NH_4Cl	74.61

[a]Measured with a Yellow Springs Model 5331 oxygen electrode.

All of the studies reviewed above are consistent with the idea that intracellular pH controls metabolism and motility. Consider first the repressed sperm in undiluted semen. If the seminal fluid were either low in Na^+ (below 0.25 mM) or low in pH (below 6.5) repression would be expected. These possibilities have recently been eliminated by direct measurements which show that the ionic composition of seminal fluid is surprisingly similar to sea water (0.44 M Na^+, pH 7.4).[10] To provide an alternative explanation, it is necessary to go back to the early studies with undiluted semen which suggested that the sperm are repressed by CO_2 anoxia. How CO_2 could generate protons and maintain a low pH_i is shown in Figure 3. CO_2 diffuses freely across the plasma membrane. Once inside a cell, and through catalysis by the enzyme carbonic anhydrase, it could combine with water to form carbonic acid which would immediately dissociate to free protons and bicarbonate ions in the measured pH_i range (6.5–6.8). Replacement of CO_2 with O_2, a treatment which stimulates motility in undiluted semen,[3] could raise the pH_i in two ways. It could simply reverse all of the equilibria or it could allow Na^+-H^+ exchange to proceed.

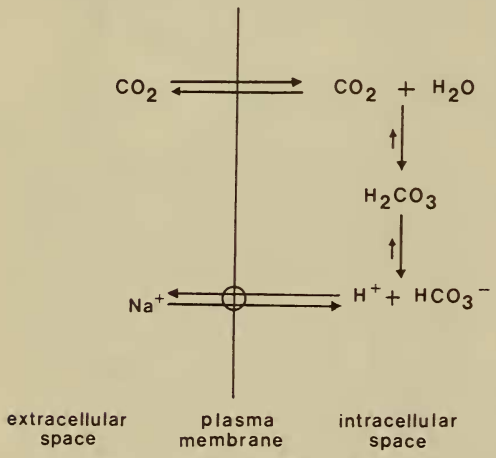


Fig. 3. The proposed scheme for the generation of intracellular protons by extracellular CO_2 and the release of intracellular protons by Na^+-H^+ exchange. See explanation in text.

Now consider the sperm upon suspension in sea water. Extracellular CO_2 levels are reduced, Na^+ is present, O_2 is provided for respiration, and metabolism and motility are stimulated. Again, the pH_i could be raised through a simple reversal of the equilibria. The results presented here, however, show that extracellular Na^+ is required to remove the protons, through Na^+-H^+ exchange. A recent and interesting finding is that even after sperm have undergone activation in sea water and are actively swimming, they may be rearrested by passing CO_2 gas over the suspension.[10] It is as if the conditions in undiluted semen have been experimentally reintroduced. Upon replacing the CO_2 with air, and since normal levels of extracellular Na^+ are present, metabolism and motility are stimulated.

Activation of the Egg

Based on the number of things that the egg must do after fertilization, egg activation is necessarily more complex. Sperm are activated solely to fertilize the egg. The egg is activated to produce a new organism. This is not to say that the cause of activation is necessarily more complex; only that its effects are. The mature unfertilized sea urchin egg is metabolically quiescent and repressed, having undergone both meiotic reduction divisions and a period of in-

tense synthetic activity and storage during oogenesis. Fusion with sperm activates or derepresses the unfertilized egg from this relatively inactive state, initiating a series of events which eventually results in cell division and development of the embryo. A chronological list of these events is shown in Figure 4. Occurring within the first minute of sperm-egg fusion are those events termed "early" which include a Na^+-dependent depolarization of the egg plasma membrane,[11] the cortical reaction,[12] and increased respiration.[13] Beginning five minutes after sperm-egg fusion are the "late" events which include development of K^+-conductance,[11,14] increased permeability to phosphate,[15] nucleosides,[16] and amino acids,[17] increased protein synthesis,[18] and initiation of DNA synthesis.[19]

Knowing the times at which these events are stimulated has provided great insight into the activation process. For example, it was first proposed by Mazia[20] and since confirmed by numerous investigators[21–24] that treatment of unfertilized eggs with millimolar concentrations of NH_4^+ bypasses the early events and selectively turns on the late events. An interesting and important aspect of this activation is that these eggs may be fertilized at any time after treatment and by this definition, remain unfertilized. Subsequent fertilization of these eggs stimulates the early events. It is as though the total activation of the egg is under control of two regulatory master switches, each activating a group of events, and the order in which the switches are thrown may be reversed.

It has become increasingly clear that the switch for the late events is the same switch implicated in the activation of sperm, increased pH_i. The fact that NH_4^+ is a stimulator is itself evidence for this idea since it could experimentally raise pH_i through the same transmembrane equilibrium. Direct measurements have now confirmed this effect.[25–28] The means by which this switch is thrown is apparently also similar to that in sperm. Chambers[29] first reported a Na^+-re-

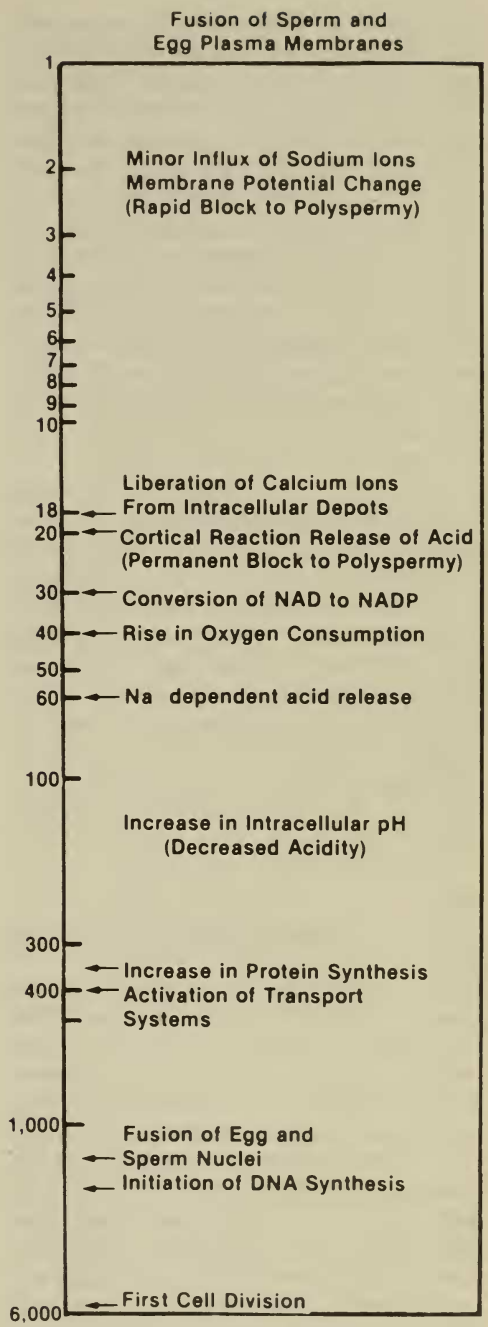


Fig. 4. Chronological list of events occurring in the sea urchin egg following sperm-egg fusion. Time is indicated logarithmically in seconds. Modified after Epel.[33]

quirement for activation and soon thereafter J Johnson *et al.*[30] reported that this requirement is for acid release. Beginning about one minute after insemination and lasting approximately four minutes, fertilized eggs release acid into the surrounding sea water. For a two percent suspension of eggs (2.0 ml packed eggs suspended in 100 ml sea water) it is detected as a drop in pH from 8.0 to 7.6.[30] This release is coupled with a measurable increase in pH_i.[25,28,30] As for sperm the effect of NH_4^+ may be interpreted as an experimental bypass of normal Na^+-H^+ exchange and a direct increase of pH_i. In the egg the effect is a turnon of the late events.

What then is the switch for the early events? This is perhaps the more important question since it would be more immediately related to the overall initiating event, the fusion of sperm and egg plasma membranes, and would lead to increased pH_i. Referring to Figure 4, it is noted that the first detectable response of the egg to the fertilizing spermatozoon is a Na^+-dependent depolarization of the egg plasma membrane. This response is due to a minor influx of Na^+ which causes the electrical charge inside the egg to go positive relative to the outside.[11] The depolarization itself is apparently not the switch, because direct injection of a depolarizing current through an intracellular electrode does not activate the unfertilized egg.[31] Instead it appears to function independently as a rapid and transient block to polypermy,[31] i.e., the fertilizing spermatozoon induces the depolarization preventing supernumerary sperm from entering the egg until more permanent blocks are established. Neither does the minor influx of Na^+ appear to be the switch, because upon stimulating sperm motility in ONa^+-SW (described in the preceding section) in the presence of eggs, fertilization and activation of the early events proceed.[6] These eggs are however polyspermic because the depolarization is prevented in the absence of Na^+.[6]

The current idea, and one that has accumulated a convincing body of evidence (reviewed by Jaffe[32] and by Epel[33]), is that a transient increase in the amount of intracellular free calcium ions (Ca^{2+}) is the switch for the early events. Mazia[34] first reported this change in 1937 and it has since been confirmed by Nakamura and Yasumasu[35] and Steinhardt *et al.*[36] The two crucial questions which these findings have raised are (1) is the increase a cause of activation, and (2) if so, how is the increase achieved. Both of these questions were approached in an important study by Steinhardt and Epel[37] using the calcium ionophore A23187 which selectively permeabilizes membranes to Ca^{2+}. They showed that treatment of unfertilized eggs with A23187 stimulated virtually all of the events normally stimulated by fertilization. Unlike ammonia-treated eggs, ionophore-treated eggs are not fertilizable because the cortical reaction, an early event, is stimulated and establishes a permanent block to polyspermy. This reaction involves a massive reorganization of the egg surface and is readily observable as the fertilization coat lifts away from the egg plasma membrane and forms a hard inpenetrable barrier to sperm.[12] Vacquier[38] has presented evidence that the cortical reaction is stimulated directly by increased levels of Ca^{2+}. An important finding related to the second question, how increased Ca^{2+} is achieved, is that activation will proceed in Ca^{2+}-free sea water.[37] This finding eliminates the need for Ca^{2+}-influx and has led to the idea that unfertilized eggs contain a store of sequestered Ca^{2+} available for release upon fertilization. The nature of this sequestration remains unknown. Since the ionophore works by causing selective permeability across membranes, one may postulate that Ca^{2+} resides in some membrane-bound organelle. Alternatively or additionally, Ca^{2+} could be bound directly to molecules within the egg. Calcium binding proteins have been reported in various types of cells and tissues[39] and Nakamura and Yasumasu[35] have provided evidence for one in sea urchin eggs.

Given the two regulatory master switches are increased intracellular free Ca^{2+} for the early events and increased pH_i for the late events, certain predictions are possible. Treatment of unfertilized eggs with NH_4^+ was shown to bypass the Ca^{2+}-requiring early events and selectively turn on the pH -dependent late events. The reciprocal result, a turnon of the early events with an arrest of the late events should be possible by increasing intracellular Ca^{2+} under conditions which prevent increased pH_i. Such a situation has been experimentally imposed in fertilized eggs transferred to ONa^+-SW, and in unfertilized eggs treated with A23187 in ONa^+-SW. Here fertilization coats are raised indicating that the Ca^{2+} increase has occurred but the eggs remain arrested because Na^+-H^+ exchange and increased pH_i are prevented. Upon addition of Na^+, acid release, increased pH_i and stimulation of the late events proceed.[30,40,41] Raising the pH of the ONa^+-SW to 9.0 with KOH or adding 0.01 M NH_4^+ also stimulate the late events in these eggs.[30,42] These experiments are analogous to those described for the activation of sperm metabolism and motility on ONa^+-SW.

Another prediction is possible for eggs fertilized in ONa^+-SW by NH_4^+-activated sperm. In this situation sperm motility has been activated by NH_4^+, and fertilization proceeds in ONa^+-SW. Since NH_4^+ is present the pH_i in the egg should also be raised and allow for complete activation. This result has been reported.[6] Apparently, increased pH_i in both the sperm and egg has been experimentally induced under conditions which prevent Na^+-H^+ exchange. A notable difference in these eggs is that instead of dividing normally into two cells at first division, they divide into many cells. The reason for this abnormality is that these eggs are polyspermically fertilized under the Na^+-free conditions imposed. The rapid block to polyspermy has been bypassed.

The results described here leave little doubt that the early events are experimentally separable from the late events. The problem that remains is to determine how they are related to one another during normal uninterrupted fertilization. Perhaps the solution is already evident. The early events accomplish two things. (1) They prevent polyspermy, ensuring that normal development will ensue, and (2) they result in a massive reorganization of the egg surface (the cortical reaction), allowing the egg to interact in new ways with its environment. The important interaction is Na^+-H^+ exchange which begins on a time course consistent with the completion of the cortical reaction. The interpretation is that the early events prepare the egg to undergo Na^+-H^+ exchange, increased pH_i, and activation of the late events by causing the proper surface changes. Viewed in this way it is easier to understand how the unfertilized egg remains suppressed in a medium fully capable of supporting activation. It does so by sequestering intracellular stores of Ca^{2+} and preventing the surface changes from occurring. It is also easier to understand how the fertilizing spermatozoon triggers the activation process. It does so by stimulating Ca^{2+}-release.

Summary and Implications for Future Study

As reviewed above, evidence has accumulated that Na^+-H^+ exchange and increased pH_i result in the activations of both the sperm and egg in the sea urchin. In the sperm these changes occur spontaneously upon dilution of semen in sea water. In the egg they are stimulated indirectly by the fertilizing spermatozoon through a transient increase of intracellular Ca^{2+}. It is often argued that the common trigger, increased pH_i, would be too simple and too nonspecific to explain the activation of cells as different as sperm and eggs. These arguments are based on misunderstandings. Increased pH_i is only the trigger. How each cell responds to it is determined by

how each cell is genetically and differentially programmed to respond to it. The spermatozoon responds by becoming motile. The egg responds by reorganizing, assembling, and synthesizing all of the materials needed for cell division and development of the embryo. Specificity is necessarily maintained in the response.

What is now obviously needed is to determine how universally applicable these findings with sea urchin gametes are to other cells which undergo activation. For reasons mentioned earlier, studies with sea urchin gametes have far exceeded those in other cell systems, especially in terms of the roles of ions in cellular activation. There are however studies which would suggest that various elements of the story in sea urchin gametes are present in the activations of other cells. Wong *et al.*[43] have recently presented evidence for Na^+-H^+ exchange and increased pH_i in the activation of sperm motility in the rat. The Ca^{2+}-ionophore A23187 has been shown to activate many different types of eggs,[44] as well as resting lymphocytes in tissue culture.[45] An increase in the pH_i of frog eggs after fertilization has been reported.[46] If one accepts the idea that cell surface changes are required for promoting ionic exchange and cellular activation as proposed here for the egg, there is an enormous body of work available correlating functional and visible changes of the cell surface with changes in the metabolic states of virtually every type of cell studied.[47] The importance of the story in the sea urchin eggs is that a link has been provided between the cell surface changes and changes inside the cell.

Acknowledgments

The author's work is supported by a grant from the National Science Foundation (No. PCM 7923487).

References Cited

1. **Gray, J.** (1928). The effect of dilution on the activity of spermatozoa. J. Exp. Biol., **5:** 337–344.
2. **Hayashi, T.** (1946). Dilution medium and survival of the spermatozoa of *Arbacia punctulata*. II. Effect of medium on respiration. Biol. Bull., **90;** 177–187.
3. **Rothschild, Lord.** (1948). The physiology of sea urchin spermatozoa. Lack of movement in semen. J. Exp. Biol., **25:** 344–352.
4. **Ohtake, H.** (1976). Respiratory behavior of sea urchin spermatozoa. I. Effect of pH and egg water on the respiratory rate. J. Exp. Zool., **198:** 303–311.
5. **Schackman, R. W., E. M. Eddy and B. M. Shapiro.** (1978). The acrosome reaction of *Strongylocentrotus purpuratus* sperm: ion requirements and movements. Dev. Biol., **65:** 483–495.
6. **Nishioka, D. and N. Cross.** (1978). The role of external sodium in sea urchin fertilization. In: *Cell Reproduction.* E. R. Dirksen, D. Prescott and C. F. Fox, eds., Academic Press, New York, pp. 403–413.
7. **Lee, H. C., S. Schuldiner, C. Johnson and D. Epel.** (1980). Sperm motility initiation: changes in intracellular pH, Ca^{+2} and membrane potential. J. Cell Biol., **87:** 39a.
8. **Lee, H. C., J. G. Forte and D. Epel.** (1982). The use of fluorescent amines for the measurement of pH_i: applications in liposomes, gastric microsomes, and sea urchin gametes. In: *Intracellular pH: Its Measurement, Regulation, and Utilization in Cellular Processes.* R. Nuccitelli and D. W. Deamer, eds., Alan R. Liss Inc., New York.
9. **Christen, R., R. W. Schackman and B. M. Shapiro.** (1981). Coupling of sperm respiration, motility, and intracellular pH (pH_i) in *Strongylocentrotus purpuratus.* J. Cell Biol., **91:** 174a.
10. **Johnson, C. H.** (1981). Activation of echinoderm gametes: the role of intracellular pH. Ph.D. Dissertation, Stanford University, Stanford, California.
11. **Steinhardt, R. A., L. Lundin and D. Mazia.** (1971). Bioelectric responses of the echinoderm egg to fertilization. Proc. Nat. Acad. Sci. USA, **68:** 2426–2430.
12. **Runnstrom, J.** (1966). The vitelline membrane and cortical particles in sea urchin eggs and their function in maturation and fertilization. Advan. Morphogen. **5:** 221–325.
13. **Epel, D.** (1964). Simultaneous measurement of TPNH formation and respiration following fertilization of the sea urchin egg. Biochem. Biophys. Res. Commun., **17:** 69–73.
14. **Steinhardt, R. A., S. Shen and D. Mazia.** (1972). Membrane potential, membrane resistance and an energy requirement for the development of potassium conductance in the fertilization reaction of echinoderm eggs. Exp. Cell Res., **72:** 195–203.
15. **Whiteley, A. H. and E. L. Chambers.** (1966). Phosphate transport in fertilized sea urchin eggs. II. Effects of metabolic inhibitors and studies on differentiation. J. Cell Physiol., **68:** 309–324.
16. **Piatigorsky, J. and A. H. Whiteley.** (1965). A

change in permeability and uptake of ^{14}C-uridine in response to fertilization in *Strongylocentrotus purpuratus* eggs. Biochim. Biophys. Acta, **108:** 404–418.

17. **Epel, D.** (1972). Activation of an Na^+-dependent amino acid transport system upon fertilization of sea urchin eggs. Exp. Cell Res., **72:** 74–89.
18. **Epel, D.** (1967). Protein synthesis in sea urchin eggs: a "late" response to fertilization. Proc. Nat. Acad. Sci. USA, **57:** 899–906.
19. **Hinegardner, R. T., B. Rao and D. E. Feldman.** (1964). The DNA synthetic period during early development of the sea urchin egg. Exp. Cell Res., **36:** 53–61.
20. **Mazia, D.** (1974). Chromosome cycles turned on in unfertilized sea urchin eggs after exposure to NH_4OH. Proc. Nat. Acad. Sci. USA, **71:** 690–693.
21. **Mazia, D. and A. Ruby.** (1974). DNA synthesis turned on in unfertilized sea urchin eggs by treatment with NH_4OH. Exp. Cell Res., **85:** 167–172.
22. **Steinhardt, R. A. and D. Mazia.** (1973). Development of K-conductance and membrane potentials in unfertilized sea urchin eggs after exposure to NH_4OH. Nature, **241:** 400–401.
23. **Epel, D., R. Steinhardt, T. Humphreys and D. Mazia.** (1974). An analysis of the partial metabolic derepression of sea urchin eggs by ammonia; the existence of independent pathways in the program of activation at fertilization. Dev. Biol., **40:** 245–255.
24. **Nishioka, D. and D. Mazia.** (1977). The phosphorylation of thymidine and the synthesis of histones in ammonia-treated eggs and egg fragments of the sea urchin. Cell Biol. Internat. Rep., **1:** 23–30.
25. **Shen, S. S. and R. A. Steinhardt.** (1978). Direct measurement of intracellular pH during metabolic derepression at fertilization and ammonia activation of the sea urchin egg. Nature, **272:** 253–254.
26. **Winkler, M. M. and J. L. Grainger.** (1978). Mechanism of action of NH_4Cl and other weak bases in the activation of sea urchin eggs. Nature, **273:** 536–538.
27. **Grainger, J. L., M. M. Winkler, S. S. Shen and R. A. Steinhardt.** (1979). Intracellular pH controls protein synthesis rate in the sea urchin egg and early embryo. Dev. Biol., **68:** 396–406.
28. **Johnson, C. H. and D. Epel.** (1981). Intracellular pH of sea urchin eggs measured by the dimethyloxazolidinedione (DMO) method. J. Cell Biol., **89:** 284–291.
29. **Chambers, E. L.** (1976). Na is essential for activation of the inseminated sea urchin egg. J. Exp. Zool., **197:** 149–154.
30. **Johnson, J. D., D. Epel and M. Paul.** (1976). Intracellular pH and activation of sea urchin eggs after fertilization. Nature, **262:** 661–664.
31. **Jaffe, L. A.** (1976). Fast block to polyspermy in sea urchin eggs is electically mediated. Nature, **261:** 68–71.
32. **Jaffe, L. F.** (1980). Calcium explosions as triggers of development. Annals New York Acad. Sci., **339:** 86–101.
33. **Epel, D.** (1980). Ionic triggers in the fertilization of sea urchin eggs. Annals New York Acad. Sci., **339:** 74–85.
34. **Mazia, D.** (1937). The release of calcium in *Arbacia* eggs on fertilization. J. Cell. Comp. Physiol., **10:** 291–308.
35. **Nakamura, M. and I. Yasumasu.** (1974). Mechanism for increase of free calcium in fertilized sea urchin eggs. A method for estimating intracellular concentration of free calcium. J. Gen. Physiol., **63:** 374–388.
36. **Steinhardt, R. A., R. Zucker and G. Schatten.** (1977). Intracellular calcium release at fertilization in the sea urchin egg. Dev. Biol., **58:** 185–196.
37. **Steinhardt, R. A. and D. Epel.** (1974). Activation of sea urchin eggs by calcium ionophore. Proc. Nat. Acad. Sci. USA, **71:** 1915–1919.
38. **Vacquier, V. D.** (1975). The isolation of intact cortical granules from sea urchin eggs: calcium ions trigger granule discharge. Dev. Biol., **43:** 62–74.
39. **Siegel, F. L.** (1973). Calcium-binding proteins. In: *Structure and Bonding, Vol. 17.* J. D. Dunitz, P. Hemmerich, J. A. Ibers, C. K. Jorgensen, J. B. Neilands, D. Reinen, R. J. P. Williams, eds., Springer-Verlag, New York.
40. **Nishioka, D. and N. F. McGwin.** (1980). Relationships between the release of acid, the cortical reaction, and the increase of protein synthesis in sea urchin eggs. J. Exp. Zool., **212:** 215–223.
41. **Nishioka, D. and L. S. Magagna.** (1981). Increased uptake of thymidine in the activation of sea urchin eggs: specificity of uptake and dependence on internal pH, the cortical reaction, and external sodium. Exp. Cell Res., **133:** 363–372.
42. **Nishioka, D. and D. Epel.** (1977). Intracellular pH and the activation of sea urchin eggs at fertilization. J. Cell Biol., **75:** 40a.
43. **Wong, P. Y. D., W. M. Lee and A. Y. F. Tsang.** (1981). The effects of extracellular sodium on acid release and motility initiation in rat caudal epididymal spermatozoa in vitro. Exp. Cell Res., **131:** 97–104.
44. **Steinhardt, R. A., D. Epel, E. J. Carroll and R. Yanagimachi.** (1974). Is calcium ionophore a universal activator for unfertilized egg? Nature, **252:** 41–43.
45. **Crumpton, M. J., B. Perles and J. Auger.** (1977). Mitogen receptors and mitogen responsiveness. In: *International Cell Biology 1967–1977.* B. R. Brinkley and K. R. Porter, eds., The Rockefeller University Press, New York, pp. 119–127.
46. **Webb, D. J. and R. Nuccitelli.** (1980). The intracellular pH goes alkaline at fertilization in the *Xenopus* egg. J. Cell Biol., **87:** 137a.
47. *The Cell Surface: Mediator of Developmental Processes.* S. Subtelny and N. K. Wessells, eds., Academic Press, New York.